11. E. E. Varfolomeev *et al.*, *Immunity* **9**, 267 (1998).
12. W. C. Yeh *et al.*, *Science* **279**, 1954 (1998).
13. W. C. Yeh *et al.*, *Immunity* **12**, 633 (2000).
14. M. Woo *et al.*, *Genes Dev.* **12**, 806 (1998).
15. E. Bossy-Wetzel, D. D. Newmeyer, D. R. Green, *EMBO J.* **17**, 37 (1998).
16. J. E. Ricci, R. A. Gottlieb, D. R. Green, *J. Cell Biol.* **160**, 65 (2003).
17. J. E. Ricci *et al.*, *Cell* **117**, 773 (2004).
18. G. Hausmann *et al.*, *J. Cell Biol.* **149**, 623 (2000).
19. V. S. Marsden *et al.*, *Nature* **419**, 634 (2002).
20. S. A. Susin *et al.*, *Nature* **397**, 441 (1999).
21. C. Cande, N. Vahsen, C. Garrido, G. Kroemer, *Cell Death Differ.* **11**, 591 (2004).

22. The authors wish to thank Y. Lazebnik (Cold Spring Harbor) for caspase 7 antibody and Biogen Idec for their contribution of interleukin-2; Yale Liver Center Core Facilities for hepatocyte preparation; A. Ferrandino and A. Mohapatra for technical assistance; and F. Manzo for assistance with manuscript preparation. This work was supported in part by NIH grants K12 HD00850, 5 K12 HD01401, 1 K08 HD044580 (S.A.L.); Liver Center Grant NIDDK P30-34989 (R.A.F.); R.A.F. is an investigator of the Howard Hughes Medical Institute.

Supporting Online Material
www.sciencemag.org/cgi/content/full/311/5762/847/DC1
Materials and Methods
Figs. S1 to S6
References and Notes

19 May 2005; accepted 20 December 2005
10.1126/science.1115035

Translational Regulators Maintain Totipotency in the *Caenorhabditis elegans* Germline

Rafal Ciosk,[1*†] Michael DePalma,[1,2] James R. Priess[1,2,3]

The molecular mechanisms that maintain totipotency of the germline are not well understood. Here, we show that two conserved translational regulators, MEX-3 and GLD-1, are essential for maintaining totipotency in the *Caenorhabditis elegans* germline. In *mex-3 gld-1* mutants, germ cells transdifferentiate into various somatic cell types such as muscles or neurons. Our findings implicate RNA regulation in the maintenance of totipotency, suggest that multiple mechanisms maintain totipotency at different stages of germline development, and establish a genetically tractable model for studying the development of teratomas.

How cells maintain or lose totipotency is a major question in stem cell and germ cell research (*1*). Germ cell precursors in early *C. elegans* and *Drosophila melanogaster* embryos maintain totipotency in part by transiently inhibiting transcription (*2*). Germ cells in larval and adult gonads are transcriptionally active and presumably require different mechanisms to maintain totipotency. The *C. elegans* hermaphrodite gonad contains germ cells in a linear sequence of developmental stages: proliferating germ cells in the distal gonad, meiotic cells in the central gonad, and cells undergoing spermatogenesis (late larvae) or oogenesis (adults) in the proximal gonad (Fig. 1A) (*2*). Many events in germline development, such as the mitosis/meiosis and spermatogenesis/oogenesis switches, involve translational regulation by the GLD-1 protein (*3*–*7*). GLD-1 is expressed primarily in the central gonad (*8*) and is a member of the signal transduction and activation of RNA (STAR) family of KH-domain, RNA binding proteins that includes mammalian Quaking and Sam68 (*9*). MEX-3 is expressed in a complementary pattern (Fig. 1A) (*10*–*12*); MEX-3 is the founding member of a distinct family of proteins with two KH-domains but is otherwise dissimilar from GLD-1. Whereas MEX-3 appears to function as a translational regulator (*10*, *13*), the functions of human orthologs such as Tino [with 83% and 75% identity to the first and second KH domains of MEX-3 (*14*)] remain unknown. Recent studies have shown that animals lacking GLD-1 misexpress MEX-3 in meiotic germ cells (*10*, *12*), raising the possibility that ectopic MEX-3 activity may contribute to previously characterized *gld-1*(–) phenotypes.

We constructed and examined *mex-3(or20) gld-1(q485)* double mutants (hereafter called *mex-3 gld-1*) lacking MEX-3 and GLD-1 activities. Many nuclei in the central gonad of the double mutants did not resemble germ nuclei in the light microscope but instead resembled nuclei found in somatic tissues (Fig. 1B). Similar nuclei were observed at a low frequency in the gonads of *gld-1* adults but were not present in *mex-3* mutants ($n > 100$) (fig. S1). Using

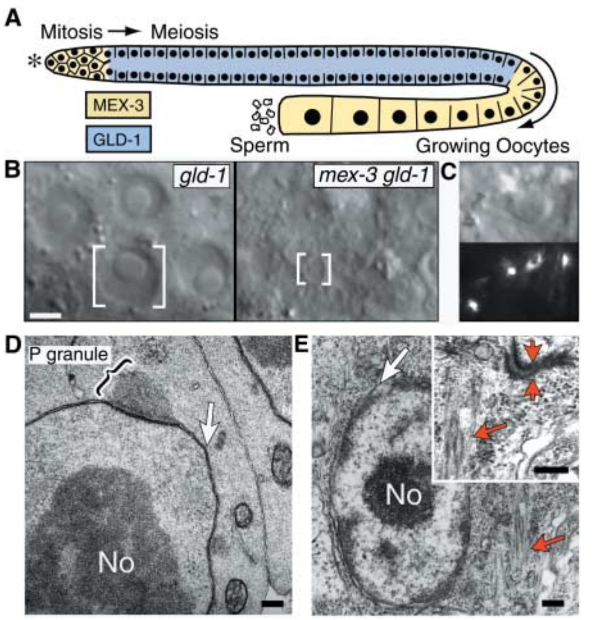

Fig. 1. Ectopic somatic cells in *mex-3 gld-1* gonads. (**A**) Diagram of wild-type gonad showing expression of MEX-3 (yellow) and GLD-1 (blue). The central region consists largely of germ nuclei at the pachytene stage of meiosis; an asterisk indicates the distal, mitotic zone. (**B**) Light micrographs of germ cells in 1-day-old *gld-1* or *mex-3 gld-1* adults; one nucleus in each gonad is bracketed. The *gld-1* germ cell nucleus resembles a wild-type germ cell nucleus (not shown) with a large nucleolus and clear nucleoplasm. *mex-3 gld-1* gonads contain some small nuclei (right) with granular nucleoplasm typical of heterochromatin in differentiated somatic cells. (**C**) The panels show light (top) and fluorescence (bottom) micrographs of a cell in a *mex-3 gld-1* gonad with birefringent-autofluorescent "gut granules." (**D**) Electron micrograph of a wild-type germ cell indicating the nucleolus (No), nuclear envelope (arrow), and a P granule (bracket). (**E**) Cell in a *mex-3 gld-1* gonad with a small nucleus, with prominent heterochromatin associated with the nuclear envelope (white arrow), and lacking P granules. The red arrow points to apparent myofilaments (magnified in the inset). An apparent adhesive junction typical of muscle cells is visible in the inset (short arrows). Scale bars: (B) 3 μm; [(D) and (E)] 0.2 μm. See (*31*).

[1]Division of Basic Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA 98109, USA. [2]Howard Hughes Medical Institute, Seattle, WA 98109, USA. [3]Molecular and Cellular Biology Program and Department of Biology, University of Washington, Seattle, WA 98195, USA.

*Present address: Friedrich Miescher Institute for Biomedical Research, 4058 Basel, Switzerland.

†To whom correspondence should be addressed. E-mail: rafal.ciosk@fmi.ch

light and electron microscopy, immunostaining, and transgenic reporters, we confirmed that the abnormal cells were differentiated somatic cells, including two types of muscle (body and pharyngeal), neurons, and intestinal cells (Fig. 1, C and E; Fig. 2, A and B; and fig. S2, A and B). The muscles contained filaments and adhesive structures resembling those found in normal muscles (Fig. 1E), expressed muscle-specific markers (Fig. 2A and fig. S2, A and B), and contracted. The neurons expressed a neuronal-specific green fluorescent protein (GFP) reporter and had extensive processes similar to normal neurons (Fig. 2, A and B, and fig. S2, A and B). Finally, some *mex-3 gld-1* gonads (35 of 134) contained cells with birefringent and autofluorescent granules characteristic of wild-type intestinal cells (Fig. 1C) (*15*). Ectopic "somatic" cells were present in *gld-1*, but not *mex-3*, single mutants at a lower frequency (1 of 143 gonads of *gld-1* mutants contained gut granules) (Fig. 2, A and C). Germ cells in wild-type gonads contain ribonucleoprotein structures called P granules that are absent from somatic cells (*2*); similar structures are uniquely associated with germ cells in a wide range of animals (*16*). The ectopic somatic cells in *mex-3 gld-1* gonads appeared to lack P granules (compare Fig. 1, D and E) and did not express the P-granule proteins PGL-1, GLH-1, and GLH-4 (*17*, *18*).

Several lines of evidence suggest that the ectopic somatic cells are transdifferentiated germ cells. The gonad primordium in a newly hatched larva contains two germ cell precursors that generate the entire germline and two cells that produce all of the other gonadal cells. When the two germ cell precursors were killed with a laser in *mex-3 gld-1* larvae, the adult gonads did not contain ectopic somatic cells (0 of 12 operated gonads and 30 of 30 control gonads contained cells with neuronal-specific GFP). Thus, the presence of germ cells is required for the ectopic somatic cells. Additional experiments showed that the ectopic somatic cells did not result from inappropriate fertilization events (table S1) or from the spontaneous activation of unfertilized oocytes. *mex-3 gld-1* gonads did not contain cells resembling mature oocytes (fig. S1), nor did they accumulate at least some oocyte-specific proteins such as OMA-1/MOE-1 (*19*). Moreover, old, unfertilized wild-type oocytes that activate spontaneously and undergo numerous rounds of DNA replication did not express muscle myosin or neuronal GFP (0 of 38 endoreplicated oocytes from 2- to 2.5-day-old wild-type adults). We found that germ cells in the central region of young adult *mex-3 gld-1* gonads (42 of 43 gonads) showed a marked reduction in the size and numbers of P granules before the appearance of ectopic somatic cells (Fig. 3A and table S1). We thus consider it likely that these aberrant germ cells are the precursors of the ectopic somatic cells that later appear in the central gonad and that lack P granules.

Somatic differentiation in *mex-3 gld-1* gonads is reminiscent of human germ cell tumors called teratomas, which contain somatic tissues such as neurons, teeth, or hair. Teratomas in male and female germlines are thought to result from distinct defects (*20*). Ovarian teratomas are the most common ovarian neoplasms and originate from germ cells that have entered, but not properly completed, meiosis (*21*, *22*). We found that the "worm teratoma" occurred only in germlines that initiated a female program of development (table S1). *C. elegans* female and male germ cells are different as early as in mitosis (*8*), but germ cell abnormalities are not apparent in *mex-3 gld-1* gonads until meiosis (Fig. 3B). Mutant germ cells at pachytene were often interspersed with aberrant germ cells containing up to 12 chromosomes (Fig. 3C). Because wild-type chromosomes pair to form six bivalents and remain paired until fertilization, the 12 chromosomes likely represent unpaired homologous chromosomes. These abnormalities suggest that defects in meiosis could contribute to transdifferentiation, although none of the meiotic mutants in *C. elegans* have been reported to undergo transdifferentiation. To address whether entry into meiosis was required for transdifferentiation, we used a *glp-1(oz112 gf)* gain-of-function mutation that forces germ cells to remain in mitosis (*23*). None of the *glp-1(gf);mex-3(RNAi)gld-1(RNAi)*

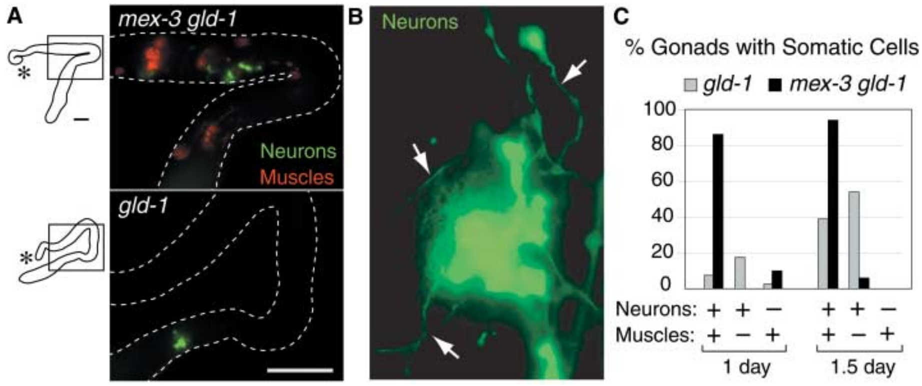

Fig. 2. MEX-3 and GLD-1 prevent transdifferentiation of germ cells. (**A**) Micrographs of 1-day-old *gld-1* or *mex-3 gld-1* adult gonads (outlined) showing clusters of apparent neurons (green) or muscles (red). Images are of boxed regions shown in the gonad diagrams at left; asterisks indicate distal tips. (**B**) High magnification of a neuronal cluster showing processes (arrows). Muscles were stained with monoclonal antibody (5.6) to myosin, and neurons expressed an *unc-119::GFP* transgene. (**C**) Quantitation of somatic differentiation in staged adult gonads (day 1: n = 40 *gld-1*, n = 29 *mex-3 gld-1*; day 1.5: n = 28 *gld-1*, n = 32 *mex-3 gld-1*).

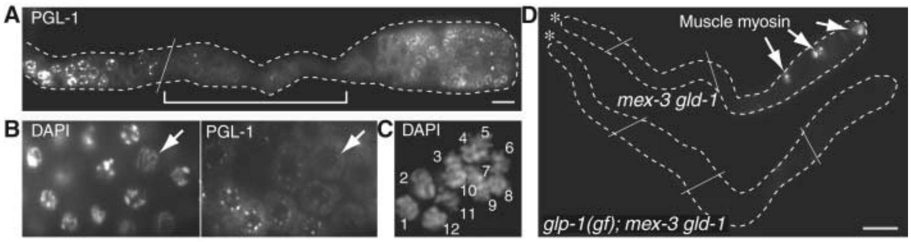

Fig. 3. P-granule loss and requirement for meiosis in transdifferentiating gonads. (**A**) A 0.5-day-old adult *mex-3 gld-1* gonad immunostained for the P-granule component PGL-1. P granules are apparent in the distal (left) and proximal (right) mitotic zones of the gonad but are diminished in the central zone (bracket). At this stage, P-granule defects were apparent in 42 of 43 gonads, although only 21 of 46 expressed the muscle factor HLH-1. (**B**) High magnification of *mex-3 gld-1* germ nuclei stained for DNA (left) and PGL-1 (right); the arrow indicates an apparent pachytene-stage nucleus lacking P granules. (**C**) High magnification of a single *mex-3 gld-1* germ nucleus with 12 chromosomes. (**D**) Gonads from a 1.5-day-old *mex-3(RNAi)gld-1(RNAi)* adult and a *glp-1(gf);mex-3(RNAi)gld-1(RNAi)* adult stained for muscle myosin. Clusters of muscles (arrows) were present in *mex-3(RNAi) gld-1(RNAi)* gonads (19 of 25 gonads) but not in *glp-1(gf);mex-3(RNAi)gld-1(RNAi)* gonads (day 1.5: n = 0 of 19; day 2: n = 0 of 57). Thin lines through gonads in this and other figures indicate boundaries of photographs used for composite images. Scale bars: (A) 20 μm; (D) 50 μm.

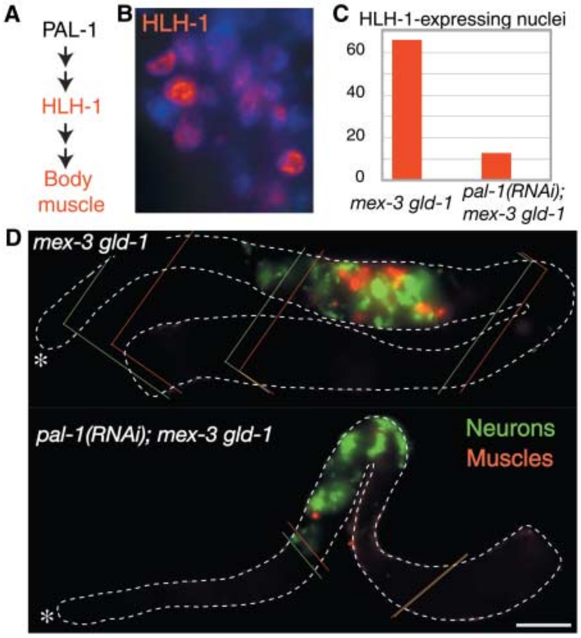

Fig. 4. PAL-1 is required for most body muscle transdifferentiation in *mex-3 gld-1* gonads. (**A**) Body muscle differentiation in normal embryogenesis involves PAL-1/Caudal and multiple downstream targets such as HLH-1/MyoD. (**B**) Inappropriate HLH-1 expression (red) in *mex-3 gld-1* germ nuclei; 4′,6′-diamidino-2-phenylindole staining shown in blue. (**C**) Quantitation of HLH-1–expressing nuclei per gonad arm; data from 12 *mex-3 gld-1* and 10 *pal-1(RNAi);mex-3 gld-1* gonads. Wild-type gonads show no detectable HLH-1 ($n > 50$). (**D**) Examples of mock-depleted (top) and PAL-1–depleted (bottom) *mex-3 gld-1* gonads. The remaining muscles in *pal-1(RNAi);mex-3 gld-1* gonads may be PAL-1–independent muscles (*24*) or may result from incomplete *pal-1(RNAi)*. Scale bar, 50 μm.

gonads contained somatic cells (Fig. 3D), suggesting that entry into meiosis is critical for transdifferentiation.

Little is known about the molecular pathways that induce teratomas (*20*). For our analysis of the *C. elegans* gonad, we focused on muscle differentiation. Most muscle precursors in normal embryogenesis are specified, in part, through a pathway that involves the transcriptional regulator PAL-1/Caudal and downstream factors such as HLH-1/MyoD (Fig. 4A) (*24*, *25*). HLH-1 was not detectable in wild-type germ nuclei but was present in large numbers of *mex-3 gld-1* germ nuclei (Fig. 4, B and C). Depletion of PAL-1 from *mex-3 gld-1* gonads caused a marked reduction in both the number of HLH-1–positive nuclei (Fig. 4C) and the number of body muscles (Fig. 4D). Thus, most of the ectopic body muscles appear to differentiate through a pathway that mimics a major muscle pathway in normal embryogenesis. Because PAL-1 and other factors that induce somatic differentiation in *C. elegans* embryos are encoded by maternally expressed mRNAs, this may explain why transdifferentiation does not occur in masculinized germlines (table S1).

Both MEX-3 and GLD-1 contribute to translational repression of *pal-1* mRNA in wild-type gonads (*11*–*13*). However, wild-type meiotic germ cells occasionally express PAL-1 without transdifferentiating (*12*). Thus, we consider it unlikely that inappropriate expression of PAL-1 is, by itself, sufficient to induce transdifferentiation. Moreover, *pal-1(RNAi);mex-3 gld-1* gonads that contain only a few body muscles contain numerous neurons and pharyngeal cells (Fig. 4D), suggesting the involvement of additional, PAL-1 independent, pathways of somatic differentiation. We propose that transdifferentiation involves both (i) the expression in germ cells of factors such as PAL-1 that normally regulate somatic differentiation in embryos and (ii) a defect that allows germ cells to respond to these factors. GLD-1 is required for wild-type meiotic germ cells to progress beyond the transcriptionally active pachytene stage of meiosis to diakinesis (*6*), where chromosomes are transcriptionally quiescent. A prolonged, aberrant pachytene stage might make germ cells sensitive to factors such as PAL-1. MEX-3 and GLD-1 regulate diverse mRNAs, and future studies should show whether specific target mRNAs have roles in transdifferentiation. For example, MEX-3/GLD-1–dependent regulation of chromatin modifiers might function in distinguishing germline and somatic states. One known GLD-1 target encodes a component of the histone H3 methyltransferase (*26*, *27*), and previous studies have shown that LET-418/Mi-2, a component of a *C. elegans* nucleosome-remodeling and histone deacetylase complex, prevents expression of germline proteins in somatic cells (*28*). The P-granule defects in *mex-3 gld-1* mutants might also contribute to transdifferentiation. P granules normally contain multiple maternally produced mRNAs and some regulators of RNA metabolism (*2*). In yeast and mammalian somatic cells, cytoplasmic structures with some similarity to P granules have a role in mRNA silencing and decay (*29*, *30*). Although no *C. elegans* mutant has been described that completely lacks P granules, P-granule defects might lead to the release and inappropriate expression of component mRNAs, resulting in transdifferentiation.

References and Notes

1. P. J. Donovan, M. P. de Miguel, *Curr. Opin. Genet. Dev.* **13**, 463 (2003).
2. G. Seydoux, T. Schedl, *Int. Rev. Cytol.* **203**, 139 (2001).
3. D. Hansen, L. Wilson-Berry, T. Dang, T. Schedl, *Development* **131**, 93 (2004).
4. E. Jan, C. K. Motzny, L. E. Graves, E. B. Goodwin, *EMBO J.* **18**, 258 (1999).
5. L. C. Kadyk, J. Kimble, *Development* **125**, 1803 (1998).
6. R. Francis, M. K. Barton, J. Kimble, T. Schedl, *Genetics* **139**, 579 (1995).
7. R. Francis, E. Maine, T. Schedl, *Genetics* **139**, 607 (1995).
8. A. R. Jones, R. Francis, T. Schedl, *Dev. Biol.* **180**, 165 (1996).
9. C. Vernet, K. Artzt, *Trends Genet.* **13**, 479 (1997).
10. R. Ciosk, M. DePalma, J. R. Priess, *Development* **131**, 4831 (2004).
11. B. W. Draper, C. C. Mello, B. Bowerman, J. Hardin, J. R. Priess, *Cell* **87**, 205 (1996).
12. D. Mootz, D. M. Ho, C. P. Hunter, *Development* **131**, 3263 (2004).
13. C. P. Hunter, C. Kenyon, *Cell* **87**, 217 (1996).
14. M. Donnini *et al.*, *J. Biol. Chem.* **279**, 20154 (2004).
15. J. S. Laufer, P. Bazzicalupo, W. B. Wood, *Cell* **19**, 569 (1980).
16. E. E. Saffman, P. Lasko, *Cell. Mol. Life Sci.* **55**, 1141 (1999).
17. I. Kawasaki *et al.*, *Cell* **94**, 635 (1998).
18. K. A. Kuznicki *et al.*, *Development* **127**, 2907 (2000).
19. M. R. Detwiler, M. Reuben, X. Li, E. Rogers, R. Lin, *Dev. Cell* **1**, 187 (2001).
20. T. M. Ulbright, *Mod. Pathol.* **18**, (Suppl 2), S61 (2005).
21. J. J. Eppig, L. P. Kozak, E. M. Eicher, L. C. Stevens, *Nature* **269**, 517 (1977).
22. D. Linder, B. K. McCaw, F. Hecht, *N. Engl. J. Med.* **292**, 63 (1975).
23. L. W. Berry, B. Westlund, T. Schedl, *Development* **124**, 925 (1997).
24. L. R. Baugh *et al.*, *Development* **132**, 1843 (2005).
25. T. Fukushige, M. Krause, *Development* **132**, 1795 (2005).
26. L. Xu, J. Paulsen, Y. Yoo, E. B. Goodwin, S. Strome, *Genetics* **159**, 1007 (2001).
27. Y. Fong, L. Bender, W. Wang, S. Strome, *Science* **296**, 2235 (2002).
28. Y. Unhavaithaya *et al.*, *Cell* **111**, 991 (2002).
29. N. Cougot, S. Babajko, B. Seraphin, *J. Cell Biol.* **165**, 31 (2004).
30. U. Sheth, R. Parker, *Science* **300**, 805 (2003).
31. Materials and methods are available as supporting material on *Science* Online.
32. We thank K. Bennett, C. Hunter, J. Kimble, M. Krause, M. Shimada, and S. Strome for reagents; R. Parker for discussion; and S. Parkhurst, L. Rohrschneider, and U. Wolke for comments on the manuscript. Some strains were provided by the Caenorhabditis Genetics Center funded by NIH. R.C. is a Leukemia and Lymphoma Society Special Fellow, and Howard Hughes Medical Institute supports M.D. and J.P.

Supporting Online Material

www.sciencemag.org/cgi/content/full/311/5762/851/DC1
Materials and Methods
Figs. S1 and S2
Table S1
References

10 November 2005; accepted 11 January 2006
10.1126/science.1122491

Translational Regulators Maintain Totipotency in the *Caenorhabditis elegans* Germline

Rafal Ciosk, Michael DePalma and James R. Priess

Science **311** (5762), 851-853.
DOI: 10.1126/science.1122491